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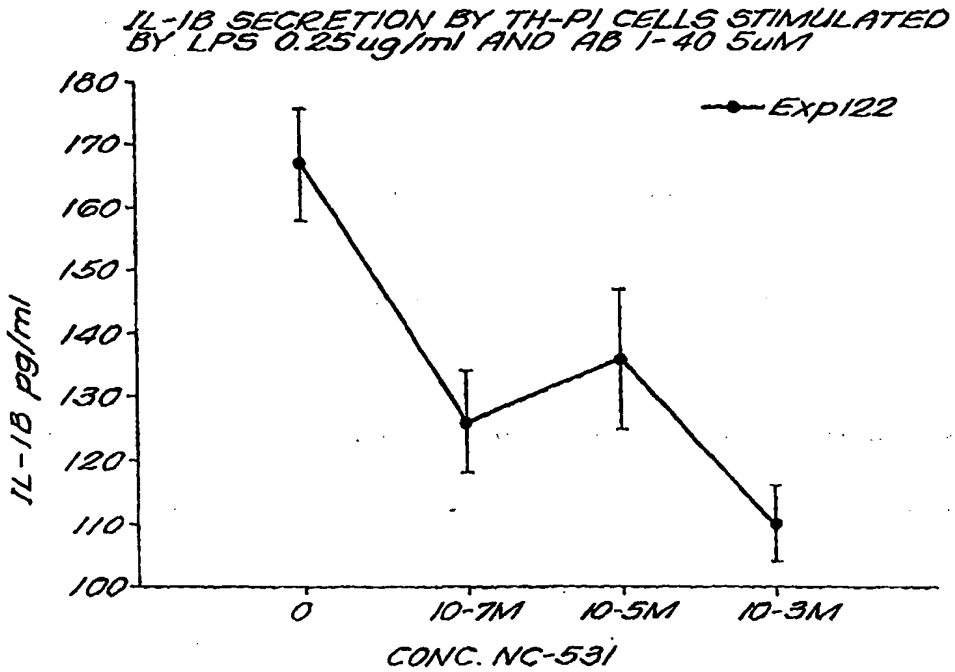

(57) Abstract

Anionic compounds, including sulfonates, are capable of blocking Aβ–induced macrophage activation (or macrophage activation induced by other amyloidogenic proteins or peptides). Such compounds can inhibit the inflammatory process, e.g., in the brain of a subject suffering from a disease characterized by Aβ deposition, such as Alzheimer's disease.

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METHOD FOR MODULATING MACROPHAGE ACTIVATION

Background of the Invention

Amyloidogenic proteins are a group of proteins which are capable of organizing into extracellular fibrillary protein deposits. These proteins, although different in nature, have a unique set of structural properties: they bind to Congo Red Staining and display an apple green birefringence when observed under polarized light.

Extracellular deposition of Aβ protein in specific regions of the brain is one of the hallmarks of Alzheimer's Disease. Aβ protein is derived from an abnormal proteolytic cleavage of the precursor protein, the βAPP. Once deposited into the brain, it forms senile plaques which have been found in greater numbers in the brains of patients with Alzheimer's Disease. It has also been shown to infiltrate cerebrovascular walls and cause angiopathy. A progressive neuronal cell loss accompanies the deposition of Aβ amyloid fibrils in senile plaques. *In vitro*, Aβ has been shown by several groups to be highly toxic to neurons. La Ferla et al. have recently shown that neuronal cells when exposed *in vitro* to soluble Aβ can become apoptotic. Once internalized, Aβ protein is stabilized and induces DNA fragmentation, which is characteristic of apoptosis. It has been shown that the 25-35 domain of the Aβ protein is responsible for such an excitotoxic activity. These results have brought scientists to consider that not only the organization of Aβ fibrils into senile plaques, which are observed late in the disease, would be detrimental to the host but that even soluble Aβ protein can induce neuronal cell loss earlier in the disease process.

Activated microglia cells have also been observed in brains of patients with Alzheimer's Disease. The activation process of these brain macrophages are thought to be responsible for the presence of inflammatory mediators in brain extracts. These mediators, e.g., inflammatory cytokines, nitric oxide and reactive oxygen intermediates, could play a major role in inducing neuronal cell toxicity. Soluble Aβ protein has recently been shown to be capable of getting internalized by microglial cells and to induce an activation process as determined by production of inflammatory mediators such as NO (Barger et al.). Giulian et al have also shown that this activation process is due to a specific domain of Aβ: the domain of residues 10-16. It is possible that this activation process is due to adherence of the protein (in particular the 10-16 domain of the Aβ protein) to the macrophage cell surface.

Summary of the Invention

It has now been discovered that certain anionic compounds, including sulfonates, are capable of blocking Aβ-induced macrophage activation (or macrophage activation induced by other amyloidogenic proteins or peptides). By interfering with the ability of Aβ to activate macrophages, such compounds can inhibit the inflammatory process, e.g., in the brain of a subject suffering from a disease characterized by Aβ deposition, such as Alzheimer's disease.

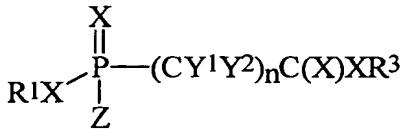
Thus, in one aspect, the invention provides a method for inhibiting macrophage activation by an amyloidogenic protein or peptide. The method comprises contacting a macrophage in the presence of an amyloidogenic protein or peptide with an anionic compound, e.g., an anionic compound of Formulae I or II, such that macrophage activation is inhibited.

In one aspect, the invention provides a method for inhibiting macrophage activation by an amyloidogenic protein or peptide. The method comprises contacting a macrophage in the presence of an amyloidogenic protein or peptide with an anionic compound, e.g., an anionic compound (e.g., of Formulae I or II), such that macrophage activation is inhibited. In one embodiment, the therapeutic compound can have the following formula (Formula I):

$$Q—[—Y^-X^+]_n$$

wherein Y^- is an anionic group at physiological pH; Q is a carrier molecule; X^+ is a cationic group; and n is an integer. The number of anionic groups ("n") is selected such that the biodistribution of the compound for an intended target site is not prevented while maintaining activity of the compound. For example, the number of anionic groups is not so great as to inhibit traversal of an anatomical barrier, such as a cell membrane, or entry across a physiological barrier, such as the blood-brain barrier, in situations where such properties are desired. In one embodiment, n is an integer between 1 and 10. In another embodiment, n is an integer between 3 and 8.

In another embodiment, the compound can be represented by the following formula (Formula II):



wherein Z is XR^2 or R^4, R^1 and R^2 are each independently hydrogen, a substituted or unsubstituted aliphatic group, an aryl group, a heterocyclic group, or a salt-forming cation; R^3 is hydrogen, lower alkyl, aryl, or a salt-forming cation; R^4 is hydrogen, lower alkyl, aryl or amino; X is, independently for each occurrence, O or S; Y^1 and Y^2 are

each independently hydrogen, halogen, alkyl, amino, hydroxy, alkoxy, or aryloxy; and n is an integer from 0 to 12.

Preferred compounds include sulfates, sulfonates, phosphates, carboxylates, and compounds which include combinations of these functional groups. Particularly preferred compounds include substituted and unsubstituted lower alkyl sulfates and sulfonates (including without limitation, 1,4-butanediol disulfate, sodium 1,5-pentanedisulfonate, taurine (sodium 2-amino-ethanesulfonate), and homotaurine (3-aminopropanesulfonic acid). Other preferred compounds include 3-(cyclohexylamino)-1-propane sulfonate, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonate, 3-(N-morpholino)propanesulfonic acid, sodium tetrahydrothiophene-1,1-dioxide-3,4-disulfate trihydrate, sodium 4-hydroxybutane-1-sulfonate, sodium 1,3,5-pentanetriol trisulfate, 2-aminoethyl hydrogen sulfate, phosphonoformic acid, phosphonoacetic acid, or indigo carmine. A preferred compound is 3-aminopropanesulfonic acid, or a salt thereof (see Example, *infra*).

In another aspect, the invention provides a method for inhibiting an inflammatory process (e.g., an inflammatory process due to the presence of, or activation of macrophages by, an amyloidogenic protein or peptide). The method comprises administering to a subject in need thereof (e.g., a subject having amyloid deposition) an effective therapeutic amount of an anionic compound, such that the inflammatory process is inhibited, e.g., by inhibition of macrophage activation by an amyloidogenic protein or peptide, such as Aβ. In a preferred embodiment, the subject is a subject suffering from Alzheimer's disease.

In certain embodiments, the anionic compound is a compound represented by Formulas I or II. Preferred compounds include sulfates, sulfonates, phosphates, carboxylates, and compounds which include combinations of these functional groups. Particularly preferred compounds include substituted and unsubstituted lower alkyl sulfates and sulfonates (including without limitation, 1,4-butanediol disulfate, sodium 1,5-pentanedisulfonate, taurine (sodium 2-amino-ethanesulfonate), and homotaurine (3-aminopropanesulfonic acid). Other preferred compounds include 3-(cyclohexylamino)-1-propane sulfonate, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonate, 3-(N-morpholino)propanesulfonic acid, sodium tetrahydrothiophene-1,1-dioxide-3,4-disulfate trihydrate, sodium 4-hydroxybutane-1-sulfonate, sodium 1,3,5-pentanetriol trisulfate, 2-aminoethyl hydrogen sulfate, phosphonoformic acid, phosphonoacetic acid, or indigo carmine. A preferred compound is 3-aminopropanesulfonic acid, or a salt thereof.

Brief Description of the Drawings

Figure 1 is a bar graph showing the effect of various conditions on nitric oxide (NO) production by macrophages in cell culture.

Figure 2 is a bar graph showing the effect of various conditions on Aβ-induced TNFα production by macrophages in cell culture.

Figure 3 is a graph showing the ability of a compound of the invention, 3-aminopropanesulfonic acid, to block or inhibit macrophage activation.

Detailed Description of the Invention

The methods of the invention provide therapeutic treatments for subjects suffering from conditions, including inflammation and neuronal cell death, e.g., subjects suffering from Alzheimer's disease or other diseases in which amyloidogenic proteins or peptides are present. By inhibiting the ability of Aβ to induce the activation process of macrophages, neuronal cell loss due to the brain inflammatory status can be slowed or prevented.

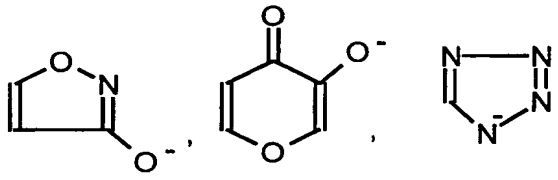
As used herein, the terms "inhibiting macrophage activation" or "inhibiting an inflammatory process" refer to decreasing, inhibiting, slowing, ameliorating, or reversing the course or degree of macrophage activation or inflammation, respectively, *in vitro* or in a subject.

In one aspect, the invention provides a method for inhibiting macrophage activation by an amyloidogenic protein or peptide. The method comprises contacting a macrophage in the presence of an amyloidogenic protein or peptide with an anionic compound, e.g., an anionic compound of Formulae I or II, such that macrophage activation is inhibited. In one embodiment, the compound can have the following formula (Formula I):

$$Q—[—Y^-X^+]_n$$

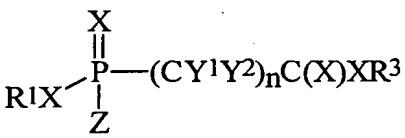
wherein Y^- is an anionic group at physiological pH; Q is a carrier molecule; X^+ is a cationic group; and n is an integer. The number of anionic groups ("n") is selected such that the biodistribution of the compound for an intended target site is not prevented while maintaining activity of the compound. For example, the number of anionic groups is not so great as to inhibit traversal of an anatomical barrier, such as a cell membrane, or entry across a physiological barrier, such as the blood-brain barrier, in situations where such properties are desired. In one embodiment, n is an integer between 1 and 10. In another embodiment, n is an integer between 3 and 8.

An anionic group of a therapeutic compound of the invention is a negatively charged moiety that, when attached to a carrier molecule, can inhibit macrophage activation by an amyloidogenic protein or peptide, or fragment thereof (e.g., Aβ or a fragment thereof). For purposes of this invention, the anionic group is negatively charged at physiological pH. Preferably, the anionic therapeutic compound mimics the structure of a sulfated proteoglycan, i.e., is a sulfated compound or a functional equivalent thereof. "Functional equivalents" of sulfates are intended to include compounds such as sulfamates as well as bioisosteres. Bioisosteres encompass both classical bioisosteric equivalents and non-classical bioisosteric equivalents. Classical and non-classical bioisosteres of sulfate groups are known in the art (see, e.g., Silverman, R.B. The Organic Chemistry of Drug Design and Drug Action, Academic Press, Inc.: San Diego, CA, 1992, pp. 19-23). Accordingly, a therapeutic compound of the invention can comprise at least one anionic group including sulfonates, sulfates, sulfamates, phosphonates, phosphates, carboxylates, and heterocyclic groups of the following formulae:



Depending on the carrier molecule, more than one anionic group can be attached thereto. When more than one anionic group is attached to a carrier molecule, the multiple anionic groups can be the same structural group (e.g., all sulfonates) or, alternatively, a combination of different anionic groups can be used (e.g., sulfonates and sulfates, etc.). It will be understood that the term "anionic group" includes salts, such as pharmaceutically acceptable salts, of an anionic group. For examples of useful compounds having anionic groups in the invention, see, e.g., U. S. Patent No. 5,643,562 (incorporated herein by reference).

In another embodiment, the compound can be represented by the following formula (Formula II):



wherein Z is XR^2 or R^4, R^1 and R^2 are each independently hydrogen, a substituted or unsubstituted aliphatic group (preferably a branched or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain; or an unsubstituted or substituted cyclic

aliphatic moiety having from 4 to 7 carbon atoms in the aliphatic ring; preferred aliphatic and cyclic aliphatic groups are alkyl groups, more preferably lower alkyl), an aryl group, a heterocyclic group, or a salt-forming cation; R^3 is hydrogen, lower alkyl, aryl, or a salt-forming cation; R^4 is hydrogen, lower alkyl, aryl or amino (including alkylamino, dialkylamino (including cyclic amino moieties), arylamino, diarylamino, and alkylarylamino); X is, independently for each occurrence, O or S; Y^1 and Y^2 are each independently hydrogen, halogen (e.g., F, Cl, Br, or I), alkyl (preferably lower alkyl), amino, hydroxy, alkoxy, or aryloxy; and n is an integer from 0 to 12 (more preferably 0 to 6, more preferably 0 or 1).

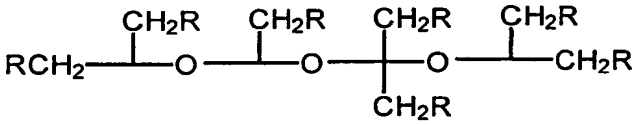
An anionic compound of the invention typically further comprises a counter cation (i.e., X^+ in the general formula: $Q—[—Y^-X^+]_n$). Cationic groups include positively charged atoms and moieties. Cationic groups include positively charged atoms and moieties. If the cationic group is hydrogen, H^+, then the compound is considered an acid, e.g., ethanesulfonic acid. If hydrogen is replaced by a metal or its equivalent, the compound is a salt of the acid. Pharmaceutically acceptable salts of the anionic compound are within the scope of the invention. For example, X^+ can be a pharmaceutically acceptable alkali metal, alkaline earth, higher valency cation (e.g., aluminum salt), polycationic counter ion or ammonium. A preferred pharmaceutically acceptable salt is a sodium salt but other salts are also contemplated within their pharmaceutically acceptable range.

Within the anionic compound, the anionic group(s) is covalently attached to a carrier molecule. Suitable carrier molecules include carbohydrates, polymers, peptides, peptide derivatives, aliphatic groups, alicyclic groups, heterocyclic groups, aromatic groups or combinations thereof. A carrier molecule can be substituted, e.g. with one or more amino, nitro, halogen, thiol or hydroxy groups.

As used herein, the term "carbohydrate" is intended to include substituted and unsubstituted mono-, oligo-, and polysaccharides. Monosaccharides are simple sugars usually of the formula $C_6H_{12}O_6$ that can be combined to form oligosaccharides or polysaccharides. Monosaccharides include enantiomers and both the D and L stereoisomers of monosaccharides. Carbohydrates can have multiple anionic groups attached to each monosaccharide moiety. For example, in sucrose octasulfate, four sulfate groups are attached to each of the two monosaccharide moieties.

As used herein, the term "polymer" is intended to include molecules formed by the chemical union of two or more combining subunits called monomers. Monomers are molecules or compounds which usually contain carbon and are of relatively low molecular weight and simple structure. A monomer can be converted to a polymer by

combination with itself or other similar molecules or compounds. A polymer may be composed of a single identical repeating subunit or multiple different repeating subunits (copolymers). Polymers within the scope of this invention include substituted and unsubstituted vinyl, acryl, styrene and carbohydrate-derived polymers and copolymers and salts thereof. In one embodiment, the polymer has a molecular weight of approximately 800-1000 Daltons. Examples of polymers with suitable covalently attached anionic groups (e.g., sulfonates or sulfates) include poly(2-acrylamido-2-methyl-1-propanesulfonic acid); poly(2-acrylamido-2-methyl-1-propanesulfonic acid-co-acrylonitrile); poly(2-acrylamido-2-methyl-1-propanesulfonic acid-co-styrene); poly(vinylsulfonic acid); poly(sodium 4-styrenesulfonic acid); and sulfates and sulfonates derived from: poly(acrylic acid); poly(methyl acrylate); poly(methyl methacrylate); and poly(vinyl alcohol); and pharmaceutically acceptable salts thereof. Examples of carbohydrate-derived polymers with suitable covalently attached anionic groups include those of the formula:



wherein R is SO_3^- or OSO_3^-; and pharmaceutically acceptable salts thereof.

Peptides and peptide derivatives can also act as carrier molecules. The term "peptide" includes two or more amino acids covalently attached through a peptide bond. Amino acids which can be used in peptide carrier molecules include those naturally occurring amino acids found in proteins such as glycine, alanine, valine, cysteine, leucine, isoleucine, serine, threonine, methionine, glutamic acid, aspartic acid, glutamine, asparagine, lysine, arginine, proline, histidine, phenylalanine, tyrosine, and tryptophan. The term amino acid further includes analogs, derivatives and congeners of naturally occurring amino acids, one or more of which can be present in a peptide derivative. For example, amino acid analogs can have lengthened or shortened side chains or variant side chains with appropriate functional groups. Also included are the D and L stereoisomers of an amino acid when the structure of the amino acid admits of stereoisomeric forms. The term "peptide derivative" further includes compounds which contain molecules which mimic a peptide backbone but are not amino acids (so-called peptidomimetics), such as benzodiazepine molecules (see e.g. James, G. L. et al. (1993) *Science* 260:1937-1942). The anionic groups can be attached to a peptide or peptide derivative through a functional group on the side chain of certain amino acids or other

suitable functional group. For example, a sulfate or sulfonate group can be attached through the hydroxyl side chain of a serine residue. A peptide can be designed to interact with a binding site for a basement membrane constituent (e.g., a GAG) in an Ab-peptide (as described above). Accordingly, in one embodiment, the peptide comprises four amino acids and anionic groups (e.g., sulfonates) are attached to the first, second and fourth amino acid. For example, the peptide can be Ser-Ser-Y-Ser, wherein an anionic group is attached to the side chain of each serine residue and Y is any amino acid. In addition to peptides and peptide derivatives, single amino acids can be used as carriers in the anionic compound of the invention. For example, cysteic acid, the sulfonate derivative of cysteine, can be used.

The term "aliphatic group" is intended to include organic compounds characterized by straight or branched chains, typically having between 1 and 22 carbon atoms. Aliphatic groups include alkyl groups, alkenyl groups and alkynyl groups. In complex structures, the chains can be branched or cross-linked. Alkyl groups include saturated hydrocarbons having one or more carbon atoms, including straight-chain alkyl groups and branched-chain alkyl groups. Such hydrocarbon moieties may be substituted on one or more carbons with, for example, a halogen, a hydroxyl, a thiol, an amino, an alkoxy, an alkylcarboxy, an alkylthio, or a nitro group. Unless the number of carbons is otherwise specified, "lower aliphatic" as used herein means an aliphatic group, as defined above (e.g., lower alkyl, lower alkenyl, lower alkynyl), but having from one to six carbon atoms. Representative of such lower aliphatic groups, e.g., lower alkyl groups, are methyl, ethyl, n-propyl, isopropyl, 2-chloropropyl, n-butyl, sec-butyl, 2-aminobutyl, isobutyl, tert-butyl, 3-thiopentyl, and the like. As used herein, the term "amino" means $-NH_2$; the term "nitro" means $-NO_2$; the term "halogen" designates -F, -Cl, -Br or -I; the term "thiol" means SH; and the term "hydroxyl" means -OH. Thus, the term "alkylamino" as used herein means an alkyl group, as defined above, having an amino group attached thereto. The term "alkylthio" refers to an alkyl group, as defined above, having a sulfhydryl group attached thereto. The term "alkylcarboxyl" as used herein means an alkyl group, as defined above, having a carboxyl group attached thereto. The term "alkoxy" as used herein means an alkyl group, as defined above, having an oxygen atom, attached thereto. Representative alkoxy groups include methoxy, ethoxy, propoxy, tert-butoxy and the like. The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous to alkyls, but which contain at least one double or triple bond respectively.

The term "alicyclic group" is intended to include closed ring structures of three or more carbon atoms. Alicyclic groups include cycloparaffins or naphthenes which are saturated cyclic hydrocarbons, cycloolefins which are unsaturated with two or more double bonds, and cycloacetylenes which have a triple bond. They do not include aromatic groups. Examples of cycloparaffins include cyclopropane, cyclohexane, and cyclopentane. Examples of cycloolefins include cyclopentadiene and cyclooctatetraene. Alicyclic groups also include fused ring structures and substituted alicyclic groups such as alkyl substituted alicyclic groups. In the instance of the alicyclics such substituents can further comprise a lower alkyl, a lower alkenyl, a lower alkoxy, a lower alkylthio, a lower alkylamino, a lower alkylcarboxyl, a nitro, a hydroxyl, $-CF_3$, -CN, or the like.

The term "heterocyclic group" is intended to include closed ring structures in which one or more of the atoms in the ring is an element other than carbon, for example, nitrogen, or oxygen. Heterocyclic groups can be saturated or unsaturated and heterocyclic groups such as pyrrole and furan can have aromatic character. They include fused ring structures such as quinoline and isoquinoline. Other examples of heterocyclic groups include pyridine and purine. Heterocyclic groups can also be substituted at one or more constituent atoms with, for example, a halogen, a lower alkyl, a lower alkenyl, a lower alkoxy, a lower alkylthio, a lower alkylamino, a lower alkylcarboxyl, a nitro, a hydroxyl, $-CF_3$, -CN, or the like.

The term "aromatic group" is intended to include unsaturated cyclic hydrocarbons containing one or more rings. Aromatic groups include 5- and 6-membered single-ring groups which may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. The aromatic ring may be substituted at one or more ring positions with, for example, a halogen, a lower alkyl, a lower alkenyl, a lower alkoxy, a lower alkylthio, a lower alkylamino, a lower alkylcarboxyl, a nitro, a hydroxyl, $-CF_3$, -CN, or the like.

In a preferred embodiment of the method of the invention, the anionic compound administered to the subject is comprised of at least one sulfonate group covalently attached to a carrier molecule, or a pharmaceutically acceptable salt thereof. Accordingly, an anionic compound can have the structure:

$$Q—[—SO_3^-X^+]_n$$

wherein Q is a carrier molecule; X^+ is a cationic group; and n is an integer. Suitable carrier molecules and cationic groups are those described hereinbefore. The number of

sulfonate groups ("n") is selected such that the biodistribution of the compound for an intended target site is not prevented while maintaining activity of the compound as discussed earlier. In one embodiment, n is an integer between 1 and 10. In another embodiment, n is an integer between 3 and 8. As described earlier, an anionic compound with multiple sulfonate groups can have the sulfonate groups spaced such that the compound interacts optimally with an HSPG binding site within the Ab peptide.

In preferred embodiments, the carrier molecule for a sulfonate(s) is a lower aliphatic group (e.g., a lower alkyl, lower alkenyl or lower alkynyl), a heterocyclic group, a disaccharide, a polymer or a peptide or peptide derivative. Furthermore, the carrier can be substituted, e.g. with one or more amino, nitro, halogen, thiol or hydroxy groups. In certain embodiments, the carrier molecule for a sulfonate(s) is an aromatic group.

Examples of suitable sulfonated polymeric anionic compounds include poly(2-acrylamido-2-methyl-1-propanesulfonic acid); poly(2-acrylamido-2-methyl-1-propanesulfonic acid-co-acrylonitrile); poly(2-acrylamido-2-methyl-1-propanesulfonic acid-co-styrene); poly(vinylsulfonic acid); poly(sodium 4-styrenesulfonic acid); a sulfonic acid derivative of poly(acrylic acid); a sulfonic acid derivative of poly(methyl acrylate); a sulfonic acid derivative of poly(methyl methacrylate); and a sulfonate derivative of poly(vinyl alcohol); and pharmaceutically acceptable salts thereof.

A preferred sulfonated polymer is poly(vinylsulfonic acid) (PVS) or a pharmaceutically acceptable salt thereof, preferably the sodium salt thereof. In one embodiment, PVS having a molecular weight of about 800-1000 Daltons is used. PVS may be used as a mixture of stereoisomers or as a single active isomer.

A preferred sulfonated disaccharide is a fully or partially sulfonated sucrose, or pharmaceutically acceptable salt thereof, such as sucrose octasulfonate. Other sulfonated saccharides include 5-deoxy-1,2-O-isopropylidene-α-D-xylofuranose-5-sulfonic acid (XXIII, shown as the sodium salt).

Preferred lower aliphatic sulfonated anionic compounds include ethanesulfonic acid; 2-aminoethanesulfonic acid (taurine); cysteic acid (3-sulfoalanine or a-amino-b-sulfopropionic acid); 1-propanesulfonic acid; 1,2-ethanedisulfonic acid; 1,3-propanedisulfonic acid; 1,4-butanedisulfonic acid; 1,5-pentanedisulfonic acid; and 4-hydroxybutane-1-sulfonic acid; and pharmaceutically acceptable salts thereof. Other aliphatic sulfonated anionic compounds include 1-butanesulfonic acid, 2-propanesulfonic acid, 3-pentanesulfonic acid, 4-heptanesulfonic acid, 1-decanesulfonic acid; and pharmaceutically acceptable salts thereof. Sulfonated substituted aliphatic anionic compounds include 3-amino-1-propanesulfonic acid, 3-hydroxypropanesulfonic

acid sulfate, 1,7-dihydroxy-4-heptanesulfonic acid; and pharmaceutically acceptable salts thereof. Yet other sulfonated compounds include 2-[(4-pyridinyl)amido]ethanesulfonic acid, and pharmaceutically acceptable salts thereof.

Preferred heterocyclic sulfonated anionic compounds include 3-(N-morpholino)propanesulfonic acid; and tetrahydrothiophene-1,1-dioxide-3,4-disulfonic acid; and pharmaceutically acceptable salts thereof.

Aromatic sulfonated anionic compounds include 1,3-benzenedisulfonic acid, 2,5-dimethoxy-1,4-benzenedisulfonic acid, 4-amino-3-hydroxy-1-naphthalenesulfonic acid, 3,4-diamino-1-naphthalenesulfonic acid; and pharmaceutically acceptable salts thereof.

In another embodiment of the method of the invention, the anionic compound administered to the subject is comprised of at least one sulfate group covalently attached to a carrier molecule, or a pharmaceutically acceptable salt thereof. Accordingly, the anionic compound can have the structure:

$$Q—[—OSO_3^-X^+]_n$$

wherein Q is a carrier molecule; X^+ is a cationic group; and n is an integer. Suitable carrier molecules and cationic groups are those described hereinbefore. The number of sulfate groups ("n") is selected such that the biodistribution of the compound for an intended target site is not prevented while maintaining activity of the anionic compound as discussed earlier. In one embodiment, n is an integer between 1 and 10. In another embodiment, n is an integer between 3 and 8. As described earlier, an anionic compound with multiple sulfate groups can have the sulfate groups spaced such that the compound interacts optimally with a GAG binding site within an Ab peptide.

In preferred embodiments, the carrier molecule for a sulfate(s) is a lower aliphatic group (e.g., a lower alkyl, lower alkenyl or lower alkynyl), an aromatic group, a disaccharide, a polymer or a peptide or peptide derivative. Furthermore, the carrier can be substituted, e.g. with one or more amino, nitro, halogen, thiol or hydroxy groups.

Examples of suitable sulfated polymeric anionic compounds include poly(2-acrylamido-2-methyl-propyl sulfuric acid); poly(2-acrylamido-2-methyl-propyl sulfuric acid-co-acrylonitrile); poly(2-acrylamido-2-methyl-propyl sulfuric acid-co-styrene); poly(vinylsulfuric acid); poly(sodium 4-styrenesulfate); a sulfate derivative of poly(acrylic acid); a sulfate derivative of poly(methyl acrylate); a sulfate derivative of poly(methyl methacrylate); and a sulfate derivative of poly(vinyl alcohol); and pharmaceutically acceptable salts thereof.

A preferred sulfated disaccharide is sucrose octasulfate or pharmaceutically acceptable salt thereof. Other sulfated saccharides include the acid form of methyl-α-D-glucopyranoside 2,3-disulfate, methyl 4,6-O-benzylidene-α-D-glucopyranoside 2,3-disulfate, 2,3,4,3',4'-sucrose pentasulfate, 1,3:4,6-di-O-benzylidene-D-mannitol 2,5-disulfate, D-mannitol 2,5-disulfate, 2,5-di-O-benzyl-D-mannitol tetrasulfate; and pharmaceutically acceptable salts thereof.

Preferred lower aliphatic sulfated anionic compounds for use in the invention include ethyl sulfuric acid; 2-aminoethan-1-ol sulfuric acid; 1-propanol sulfuric acid; 1,2-ethanediol disulfuric acid; 1,3-propanediol disulfuric acid; 1,4-butanediol disulfuric acid; 1,5-pentanediol disulfuric acid; and 1,4-butanediol monosulfuric acid; and pharmaceutically acceptable salts thereof. Other sulfated aliphatic anionic compounds contemplated for use in the invention include the acid form of 1,3-cyclohexanediol disulfate, 1,3,5-heptanetriol trisulfate, 2-hydroxymethyl-1,3-propanediol trisulfate, 2-hydroxymethyl-2-methyl-1,3-propanediol trisulfate, 1,3,5,7-heptanetetraol tetrasulfate, 1,3,5,7,9-nonane pentasulfate; and pharmaceutically acceptable salts thereof. Other sulfated anionic compounds contemplated for use in the invention include the acid form of 2-amino-2-hydroxymethyl-1,3-propanediol trisulfate, 2-benzyloxy-1,3-propanediol disulfate, 3-hydroxypropylsulfamic acid sulfate, 2,2'-iminoethanol disulfate, N,N-bis(2-hydroxyethyl)sulfamic acid disulfate,; and pharmaceutically acceptable salts thereof.

Preferred heterocyclic sulfated anionic compounds include 3-(N-morpholino)propanesulfuric acid; and tetrahydrothiophene-1,1-dioxide-3,4-diol disulfuric acid; and pharmaceutically acceptable salts thereof.

The invention further contemplates the use of prodrugs which are converted *in vivo* to the anionic compounds used in the methods of the invention (see, e.g., R.B. Silverman, 1992, "The Organic Chemistry of Drug Design and Drug Action", Academic Press, Chp. 8). Such prodrugs can be used to alter the biodistribution (e.g., to allow compounds which would not typically cross the blood-brain barrier to cross the blood-brain barrier) or the pharmacokinetics of the anionic compound. For example, an anionic group, e.g., a sulfate or sulfonate, can be esterified, e.g, with a methyl group or a phenyl group, to yield a sulfate or sulfonate ester. When the sulfate or sulfonate ester is administered to a subject, the ester is cleaved, enzymatically or non-enzymatically, reductively or hydrolytically, to reveal the anionic group. Such an ester can be cyclic, e.g., a cyclic sulfate or sultone, or two or more anionic moieties may be esterified through a linking group. Exemplary cyclic anionic compounds include, for example, 2-sulfobenzoic acid, propane sultone, butane sultone, 1,3-butanediol cyclic sulfate, α-chloro-α-hydroxy-o-toluenesulfonic acid sultone, and 6-nitronaphth-[1,8-cd]-1,2,-

oxathiole 2,2-dioxide. In a preferred embodiment, the prodrug is a cyclic sulfate or sultone. An anionic group can be esterified with moieties (e.g., acyloxymethyl esters) which are cleaved to reveal an intermediate anionic compound which subsequently decomposes to yield the active anionic compound. In another embodiment, the prodrug is a reduced form of a sulfate or sulfonate, e.g., a thiol, which is oxidized *in vivo* to the anionic compound. Furthermore, an anionic moiety can be esterified to a group which is actively transported *in vivo*, or which is selectively taken up by target organs. The ester can be selected to allow specific targeting of the anionic compounds to particular organs, as described below for carrier moieties.

Carrier molecules useful in the anionic compounds include carrier molecules previously described, e.g. carbohydrates, polymers, peptides, peptide derivatives, aliphatic groups, alicyclic groups, heterocyclic groups, aromatic groups or combinations thereof. Suitable polymers include substituted and unsubstituted vinyl, acryl, styrene and carbohydrate-derived polymers and copolymers and salts thereof. Preferred carrier molecules include a lower alkyl group, a heterocyclic group, a disaccharide, a polymer or a peptide or peptide derivative.

Carrier molecules useful in the present invention may also include moieties which allow the anionic compound to be selectively delivered to a target organ or organs. For example, if delivery of a tanionic compound to the brain is desired, the carrier molecule may include a moiety capable of targeting the anionic compound to the brain, by either active or passive transport (a "targeting moiety"). Illustratively, the carrier molecule may include a redox moiety, as described in, for example, U.S. Patents 4,540,564 and 5,389,623, both to Bodor. These patents disclose drugs linked to dihydropyridine moieties which can enter the brain, where they are oxidized to a charged pyridinium species which is trapped in the brain. Thus, drug accumulates in the brain. Exemplary pyridine/dihdropyridine compounds of the invention include sodium 1-(3-sulfopropyl)-1,4-dihydropyridine, sodium 2-(nicotinylamido)-ethanesulfonate, and 1-(3-sulfopropyl)-pyridinium betaine. Other carrier moieties include compounds, such as amino acids or thyroxine, which can be passively or actively transported *in vivo*. An illustrative compound is phenylalanyltaurine, in which a taurine molecule is conjugated to a phenylalanine (a large neutral amino acid). Such a carrier moiety can be metabolically removed *in vivo*, or can remain intact as part of an active anionic compound. Structural mimics of amino acids (and other actively transported moieties) are also useful in the invention (e.g., 1-(aminomethyl)-1-(sulfomethyl)-cyclohexane). Other exemplary amino acid mimetics include p-(sulfomethyl)phenylalanine, p-(1,3-disulfoprop-2-yl)phenylalanine, and O-(1,3-disulfoprop-2-yl)tyrosine. Many targeting

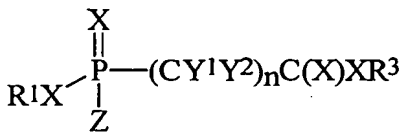
moieties are known, and include, for example, asialoglycoproteins (see, e.g. Wu, U.S. Patent 5,166,320) and other ligands which are transported into cells via receptor-mediated endocytosis (see below for further examples of targeting moieties which may be covalently or non-covalently bound to a carrier molecule). Furthermore, the anionic compounds of the invention may bind to amyloidogenic proteins, e.g., Ab peptide, in the circulation and thus be transported to the site of action.

The targeting and prodrug strategies described above can be combined to produce an anionic compound that can be transported as a prodrug to a desired site of action and then unmasked to reveal an active anionic compound. For example, the dihydropyrine strategy of Bodor (see *supra*) can be combined with a cyclic prodrug, as for example in the compound 2-(1-methyl-1,4-dihydronicotinyl)amidomethyl-propanesultone.

In one embodiment, the anionic compound in the pharmaceutical compositions is a sulfonated polymer, for example poly(2-acrylamido-2-methyl-1-propanesulfonic acid); poly(2-acrylamido-2-methyl-1-propanesulfonic acid-co-acrylonitrile); poly(2-acrylamido-2-methyl-1-propanesulfonic acid-co-styrene); poly(vinylsulfonic acid); poly(sodium 4-styrenesulfonic acid); a sulfonate derivative of poly(acrylic acid); a sulfonate derivative of poly(methyl acrylate); a sulfonate derivative of poly(methyl methacrylate); and a sulfonate derivative of poly(vinyl alcohol); and pharmaceutically acceptable salts thereof.

In another embodiment, the anionic compound in the pharmaceutical compositions is a sulfated polymer, for example poly(2-acrylamido-2-methyl-1-propanesulfuric acid); poly(2-acrylamido-2-methyl-1-propanesulfuric acid-co-acrylonitrile); poly(2-acrylamido-2-methyl-1-propanesulfuric acid-co-styrene); poly(vinylsulfuric acid); poly(sodium 4-styrenesulfate); a sulfate derivative of poly(acrylic acid); a sulfate derivative of poly(methyl acrylate); a sulfate derivative of poly(methyl methacrylate); and a sulfate derivative of poly(vinyl alcohol); and pharmaceutically acceptable salts thereof.

The anionic compound can also have the structure:

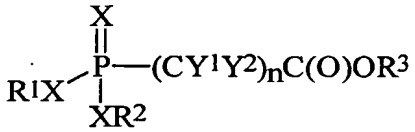


in which Z is XR^2 or R^4, R^1 and R^2 are each independently hydrogen, a substituted or unsubstituted aliphatic group (preferably a branched or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain; or an unsubstituted or substituted cyclic

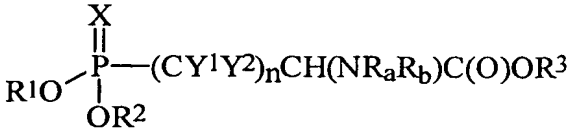
aliphatic moiety having from 4 to 7 carbon atoms in the aliphatic ring; preferred aliphatic and cyclic aliphatic groups are alkyl groups, more preferably lower alkyl), an aryl group, a heterocyclic group, or a salt-forming cation; R^3 is hydrogen, lower alkyl, aryl, or a salt-forming cation; X is, independently for each occurrence, O or S; R^4 is hydrogen, lower alkyl, aryl or amino; Y^1 and Y^2 are each independently hydrogen, halogen (e.g., F, Cl, Br, or I), lower alkyl, amino (including alkylamino, dialkylamino, arylamino, diarylamino, and alkylarylamino), hydroxy, alkoxy, or aryloxy; and n is an integer from 0 to 12 (more preferably 0 to 6, more preferably 0 or 1). These compounds are described in U.S. Application Serial No. 08/912,574, the contents of which are incorporated herein by reference.

Preferred anionic compounds for use in the invention include compounds in which both R^1 and R^2 are pharmaceutically acceptable salt-forming cations. It will be appreciated that the stoichiometry of an anionic compound to a salt-forming counterion (if any) will vary depending on the charge of the anionic portion of the compound (if any) and the charge of the counterion. In a particularly preferred embodiment, R^1, R^2 and R^3 are each independently a sodium, potassium or calcium cation. In certain embodiments in which at least one of R^1 and R^2 is an aliphatic group, the aliphatic group has between 1 and 10 carbons atoms in the straight or branched chain, and is more preferably a lower alkyl group. In other embodiments in which at least one of R^1 and R^2 is an aliphatic group, the aliphatic group has between 10 and 24 carbons atoms in the straight or branched chain. In certain preferred embodiments, n is 0 or 1; more preferably, n is 0. In certain preferred embodiments of the therapeutic compounds, Y^1 and Y^2 are each hydrogen.

In certain preferred embodiments, the anionic compound of the invention can have the structure:



in which R^1, R^2, R^3, Y^1, Y^2, X and n are as defined above. In more preferred embodiments, the anionic compound of the invention can have the structure:

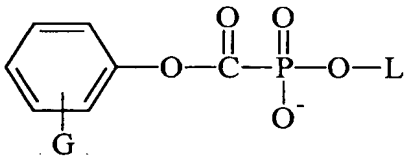


in which R^1, R^2, R^3, Y^1, Y^2, and X are as defined above, R_a and R_b are each independently hydrogen, alkyl, aryl, or heterocyclyl, or R_a and R_b, taken together with

the nitrogen atom to which they are attached, form a cyclic moiety having from 3 to 8 atoms in the ring, and n is an integer from 0 to 6. In certain preferred embodiments, R_a and R_b are each hydrogen. In certain preferred embodiments, a compound of the invention comprises an α-amino acid (or α-amino acid ester), more preferably a L-α-amino acid or ester.

The Z, Q, R^1, R^2, R^3, Y^1, Y^2 and X groups are each independently selected such that the biodistribution of the anionic compound for an intended target site is not prevented while maintaining activity of the anionic compound. For example, the number of anionic groups (and the overall charge on the therapeutic compound) should not be so great as to inhibit traversal of an anatomical barrier, such as a cell membrane, or entry across a physiological barrier, such as the blood-brain barrier, in situations where such properties are desired. For example, it has been reported that esters of phosphonoformate have biodistribution properties different from, and in some cases superior to, the biodistribution properties of phosphonoformate (see, e.g., U.S. Patent Nos. 4,386,081 and 4,591583 to Helgstrand et al., and U.S. Patent Nos. 5,194,654 and 5,463,092 to Hostetler et al.). Thus, in certain embodiments, at least one of R^1 and R^2 is an aliphatic group (more preferably an alkyl group), in which the aliphatic group has between 10 and 24 carbons atoms in the straight or branched chain. The number, length, and degree of branching of the aliphatic chains can be selected to provide a desired characteristic, e.g., lipophilicity. In other embodiments, at least one of R^1 and R^2 is an aliphatic group (more preferably an alkyl group), in which the aliphatic group has between 1 and 10 carbons atoms in the straight or branched chain. Again, the number, length, and degree of branching of the aliphatic chains can be selected to provide a desired characteristic, e.g., lipophilicity or ease of ester cleavage by enzymes. In certain embodiments, a preferred aliphatic group is an ethyl group.

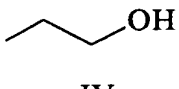
In another embodiment, the anionic compound of the invention can have the structure:



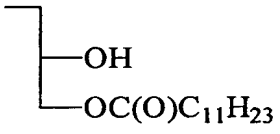
in which G represents hydrogen or one or more substituents on the aryl ring (e.g., alkyl, aryl, halogen, amino, and the like) and L is a substituted alkyl group (in certain embodiments, preferably a lower alkyl), more preferably a hydroxy-substituted alkyl or an alkyl substituted with a nucleoside base. In certain embodiments, G is hydrogen or an electron-donating group. In embodiments in which G is an electron-withdrawing

group, G is preferably an electron withdrawing group at the meta position. The term "electron-withdrawing group" is known in the art, and, as used herein, refers to a group which has a greater electron-withdrawing than hydrogen. A variety of electron-withdrawing groups are known, and include halogens (e.g., fluoro, chloro, bromo, and iodo groups), nitro, cyano, and the like. Similarly, the term "electron-donating group", as used herein, refers to a group which is less electron-withdrawing than hydrogen. In embodiments in which G is an electron donating group, G can be in the ortho, meta or para position.

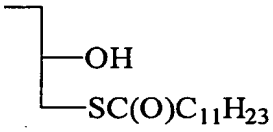
In certain preferred embodiments, L is a moiety selected from the group consisting of :



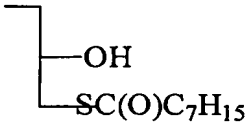
IVa



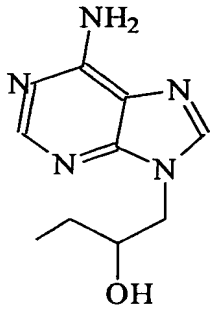
IVb



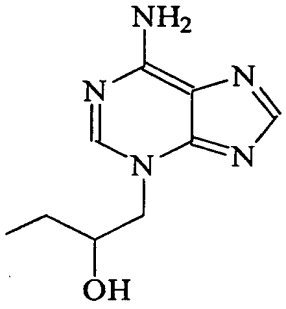
IVc



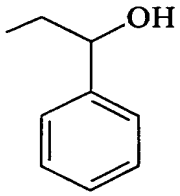
IVd



IVe



IVf



IVg

Table 1 lists data pertinent to the characterization of these compounds using art-recognized techniques.

Table 1

COMPOUND	^{31}P NMR	^{13}C NMR	FAB-MS(-)
IVa	-6.33(DMSO-d_6)	60.97 CH_2OH(d, J=6Hz) 66.76 CHOH(d, J=7.8Hz) 121.65, 121.78, 121.99, 125.71, 129.48, 129.57, 126.43 Aromatic CH 134.38 Aniline C-N 150.39 Phenyl C-O(d, J=7Hz) 171.57 P-C=O(d, J=234Hz)	245.2
IVb	-6.41(DMSO-d_6)	13.94 CH_3 22.11, 24.40, 28.56, 28.72, 28.99, 29.00, 31.30, 33.43, -$(CH_2)_{10}$- 65.03 CH_2-OC(O) 66.60 CH_2-OP(d, J=5.6Hz) 67.71 CH2-OH(d, J=6 Hz) 121.73, 121.10, 125.64, 126.57, 129.40, 129.95, Aromatic CH 134.04 Aniline C-N 150.31 Phenyl C-O 171.44 P-C=O(d, J=6.7 Hz) 172.83 O-C=O	456
IVc	-6.46(DMSO-d_6)	13.94 CH_3 22.11, 25.10, 28.68, 28.72, 28.85, 29.00, 30.76, 31.31, 32.10, -$(CH_2)_{10}$- 43.36 CH_2-S 68.43 CH_2-OH 68.43 CH-OH(d, J=6.3 Hz) 68.76 P-O-CH_2-9d, J=5.8 Hz) 121.75, 122.03, 125.62, 126.37, 129.30, 129.53, Aromatic CH 134.23 Aniline C-N 150.37 Phenyl C-O(d, J=6.7 Hz) 171.47 P-C=O(d, J=234.0 Hz) 198.47 S-C=O	471

COMPOUND	^{31}P NMR	^{13}C NMR	FAB-MS(-)
IVd	-6.61(DMSO-d_6)	13.94 CH_3 22.06, 25.14, 28.24, 28.35, 31.09, 32.14 -CH_2)$_6$- 43.40 CH_2-S 68.50 P-O-CH_2-(d, J=5.8 Hz) 68.77 CH-OH(d, 6.4 Hz) 121.78, 122.59, 125.69, 127.06, 129.43, 129.59 Aromatic CH 133.39 Aniline C-N 150.38 Phenyl C-O(d, J=6.7 Hz) 171.47 P-C=O(d, J=234.4 Hz) 198.54 S-C=O	416
IVe	-5.76(D_2O)	N/A	N/A
IVf	-7.00(DMSO-d_6)	N/A	N/A
IVg	-6.60(DMSO-D6)	70.84 CH2-OH 72.17 CH-OH 121.68, 121.79, 121.85, 125.71 127.10, 127.92, 129.36, 129.50, 129.59 Aromatic CH 134.51 Aniline C-N 142.34 Aromatic C-CH 150.37 Phenyl C-O(d, J=6.2 Hz) 171.59 P-C=O(d, J=232.6 Hz)	321

It will be noted that the structure of some of the anionic compounds of this invention includes asymmetric carbon atoms. It is to be understood accordingly that the isomers (e.g., enantiomers and diastereomers) arising from such asymmetry are included within the scope of this invention. Such isomers can be obtained in substantially pure

form by classical separation techniques and by sterically controlled synthesis. For the purposes of this application, unless expressly noted to the contrary, an anionic compound shall be construed to include both the R or S stereoisomers at each chiral center.

In certain embodiments, an anionic compound of the invention comprises a cation (i.e., in certain embodiments, at least one of R^1, R^2 or R^3 is a cation). If the cationic group is hydrogen, H^+, then the anionic compound is considered an acid, e.g., phosphonoformic acid. If hydrogen is replaced by a metal ion or its equivalent, the anionic compound is a salt of the acid. Pharmaceutically acceptable salts of the anionic compound are within the scope of the invention. For example, at least one of R^1, R^2 or R^3 can be a pharmaceutically acceptable alkali metal (e.g., Li, Na, or K), ammonium cation, alkaline earth cation (e.g., Ca^{2+}, Ba^{2+}, Mg^{2+}), higher valency cation, or polycationic counter ion (e.g., a polyammonium cation). (See, e.g., Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19). It will be appreciated that the stoichiometry of an anionic compound to a salt-forming counterion (if any) will vary depending on the charge of the anionic portion of the compound (if any) and the charge of the counterion. Preferred pharmaceutically acceptable salts include a sodium, potassium or calcium salt, but other salts are also contemplated within their pharmaceutically acceptable range.

The term "pharmaceutically acceptable esters" refers to the relatively non-toxic, esterified products of the anionic compounds of the present invention. These esters can be prepared *in situ* during the final isolation and purification of the anionic compounds or by separately reacting the purified anionic compound in its free acid form or hydroxyl with a suitable esterifying agent; either of which are methods known to those skilled in the art. Carboxylic acids and phosphonic acids can be converted into esters according to methods well known to one of ordinary skill in the art, e.g., *via* treatment with an alcohol in the presence of a catalyst. A preferred ester group (e.g., when R^3 is lower alkyl) is an ethyl ester group.

The term "alkyl" refers to the saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C_1-C_{30} for straight chain, C_3-C_{30} for branched chain), and more preferably 20 or fewer. Likewise, preferred cycloalkyls have from 4-10 carbon atoms in their ring structure, and more preferably have 4-7 carbon atoms in the ring structure.

The term "lower alkyl" refers to alkyl groups having from 1 to 6 carbons in the chain, and to cycloalkyls having from 3 to 6 carbons in the ring structure.

Moreover, the term "alkyl" (including "lower alkyl") as used throughout the specification and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfate, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. Cycloalkyls can be further substituted, e.g., with the substituents described above. An "aralkyl" moiety is an alkyl substituted with an aryl (e.g., phenylmethyl (benzyl)).

The term "alkoxy", as used herein, refers to a moiety having the structure -O-alkyl, in which the alkyl moiety is described above.

The term "aryl" as used herein includes 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, unsubstituted or substituted benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Aryl groups also include polycyclic fused aromatic groups such as naphthyl, quinolyl, indolyl, and the like. The aromatic ring can be substituted at one or more ring positions with such substituents, e.g., as described above for alkyl groups. Preferred aryl groups include unsubstituted and substituted phenyl groups.

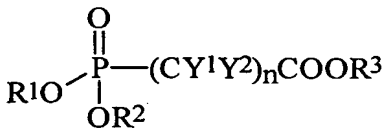
The term "aryloxy", as used herein, refers to a group having the structure -O-aryl, in which the aryl moiety is as defined above.

The term "amino," as used herein, refers to an unsubstituted or substituted moiety of the formula $-NR_aR_b$, in which R_a and R_b are each independently hydrogen, alkyl, aryl, or heterocyclyl, or R_a and R_b, taken together with the nitrogen atom to which they are attached, form a cyclic moiety having from 3 to 8 atoms in the ring. Thus, the term "amino" is intended to include cyclic amino moieties such as piperidinyl or pyrrolidinyl groups, unless otherwise stated. An "amino-substituted amino group" refers to an

amino group in which at least one of R_a and R_b, is further substituted with an amino group.

In a preferred embodiment, R^1 or R^2 can be (for at least one occurrence) a long-chain aliphatic moiety. The term "long-chain aliphatic moiety" as used herein, refers to a moiety having a straight or branched chain aliphatic moiety (e.g., an alkyl or alkenyl moiety) having from 10 to 24 carbons in the aliphatic chain, e.g., the long-chain aliphatic moiety is an aliphatic chain of a fatty acid (preferably a naturally-occurring fatty acid). Representative long-chain aliphatic moieties include the aliphatic chains of stearic acid, oleic acid, linolenic acid, and the like.

In certain embodiments, the anionic compound of the invention can have the structure:



in which R^1 and R^2 are each independently hydrogen, an aliphatic group (preferably a branched or straight-chain aliphatic moiety having from 1 to 24 carbon atoms, more preferably 10-24 carbon atoms, in the chain; or an unsubstituted or substituted cyclic aliphatic moiety having from 4 to 7 carbon atoms in the aliphatic ring), an aryl group, a heterocyclic group, or a salt-forming cation; R^3 is hydrogen, lower alkyl, aryl, or a salt-forming cation; Y^1 and Y^2 are each independently hydrogen, halogen (e.g., F, Cl, Br, or I), lower alkyl, hydroxy, alkoxy, or aryloxy; and n is an integer from 0 to 12. Preferred anionic compounds for use in the invention include compounds in which both R^1 and R^2 are pharmaceutically acceptable salt-forming cations. In a particularly preferred embodiment, R^1, R^2 and R^3 are each independently a sodium, potassium or calcium cation, and n is 0. In certain preferred embodiments of the therapeutic compounds, Y^1 and Y^2 are each hydrogen. Particularly preferred anionic compounds are salts of phosphonoformate. Trisodium phosphonoformate (foscarnet sodium or Foscavir®) is commercially available (e.g., from Astra), and its clinical pharmacology has been investigated (see, e.g., "Physician's Desk Reference", 51st Ed., pp. 541-545 (1997)).

In another embodiment, the anionic compound used in the invention can be an aminophosphonate, a biphosphonate, a phosphonocarboxylate derivative, a phosphonate derivative, or a phosphono carbohydrate.

Pharmaceutically Acceptable Formulations

In the method of the invention, the anionic compound can be administered in a pharmaceutically acceptable formulation. The present invention pertains to any pharmaceutically acceptable formulations, such as synthetic or natural polymers in the form of macromolecular complexes, nanocapsules, microspheres, or beads, and lipid-based formulations including oil-in-water emulsions, micelles, mixed micelles, synthetic membrane vesicles, and resealed erythrocytes.

In one embodiment, the pharmaceutically acceptable formulations comprise a polymeric matrix.

The terms "polymer" or "polymeric" are art-recognized and include a structural framework comprised of repeating monomer units which is capable of delivering an anionic compound, such that treatment of a targeted condition occurs. The terms also include co-polymers and homopolymers, e.g., synthetic or naturally occurring. Linear polymers, branched polymers, and cross-linked polymers are also meant to be included.

For example, polymeric materials suitable for forming the pharmaceutically acceptable formulation employed in the present invention, include naturally derived polymers such as albumin, alginate, cellulose derivatives, collagen, fibrin, gelatin, and polysaccharides, as well as synthetic polymers such as polyesters (PLA, PLGA), polyethylene glycol, poloxomers, polyanhydrides, and pluronics. These polymers are biocompatible with the nervous system, including the central nervous system, they are biodegradable within the central nervous system without producing any toxic byproducts of degradation, and they possess the ability to modify the manner and duration of anionic compound release by manipulating the polymer's kinetic characteristics. As used herein, the term "biodegradable" means that the polymer will degrade over time by the action of enzymes, by hydrolytic action and/or by other similar mechanisms in the body of the subject. As used herein, the term "biocompatible" means that the polymer is compatible with a living tissue or a living organism by not being toxic or injurious and by not causing an immunological rejection.

Polymers can be prepared using methods known in the art (Sandler, S. R.; Karo, W. *Polymer Syntheses;* Harcourt Brace: Boston, 1994; Shalaby, W.; Ikada, Y.; Langer, R.; Williams, J. *Polymers of Biological and Biomedical Significance (ACS Symposium Series 540;* American Chemical Society: Washington, DC, 1994). Polymers can be designed to be flexible; the distance between the bioactive side-chains and the length of a linker between the polymer backbone and the group can be controlled. Other suitable polymers and methods for their preparation are described in U.S. Patent Nos. 5,455,044 and 5,576,018, the contents of which are incorporated herein by reference.

The polymeric formulations are preferably formed by dispersion of the anionic compound within liquefied polymer, as described in U.S. Pat. No. 4,883,666, the teachings of which are incorporated herein by reference, or by such methods as bulk polymerization, interfacial polymerization, solution polymerization and ring polymerization as described in Odian G., Principles of Polymerization and ring opening polymerization, 2nd ed., John Wiley & Sons, New York, 1981, the contents of which are incorporated herein by reference. The properties and characteristics of the formulations are controlled by varying such parameters as the reaction temperature, concentrations of polymer and anionic compound, types of solvent used, and reaction times.

In addition to the anionic compound and the pharmaceutically acceptable polymer, the pharmaceutically acceptable formulation used in the method of the invention can comprise additional pharmaceutically acceptable carriers and/or excipients. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and anti fungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. For example, the carrier can be suitable for injection into the cerebrospinal fluid. Excipients include pharmaceutically acceptable stabilizers and disintegrants.

The anionic compound can be encapsulated in one or more pharmaceutically acceptable polymers, to form a microcapsule, microsphere, or microparticle, terms used herein interchangeably. Microcapsules, microspheres, and microparticles are conventionally free-flowing powders consisting of spherical particles of 2 millimeters or less in diameter, usually 500 microns or less in diameter. Particles less than 1 micron are conventionally referred to as nanocapsules, nanoparticles or nanospheres. For the most part, the difference between a microcapsule and a nanocapsule, a microsphere and a nanosphere, or microparticle and nanoparticle is size; generally there is little, if any, difference between the internal structure of the two. In one aspect of the present invention, the mean average diameter is less than about 45 μm, preferably less than 20 μm, and more preferably between about 0.1 and 10 μm.

In another embodiment, the pharmaceutically acceptable formulations comprise lipid-based formulations. Any of the known lipid-based drug delivery systems can be used in the practice of the invention. For instance, multivesicular liposomes (MVL), multilamellar liposomes (also known as multilamellar vesicles or "MLV"), unilamellar liposomes, including small unilamellar liposomes (also known as unilamellar vesicles or "SUV") and large unilamellar liposomes (also known as large unilamellar vesicles or "LUV"), can all be used so long as a sustained release rate of the encapsulated anionic compound can be established. In one embodiment, the lipid-based formulation can be a

multivesicular liposome system. Methods of making controlled release multivesicular liposome drug delivery systems is described in PCT Application Serial Nos. US96/11642, US94/12957 and US94/04490, the contents of which are incorporated herein by reference.

The composition of the synthetic membrane vesicle is usually a combination of phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used.

Examples of lipids useful in synthetic membrane vesicle production include phosphatidylglycerols, phosphatidylcholines, phosphatidylserines, phosphatidylethanolamines, sphingolipids, cerebrosides, and gangliosides. Preferably phospholipids including egg phosphatidylcholine, dipalmitoylphosphatidylcholine, distearoylphosphatidylcholine, dioleoylphosphatidylcholine, dipalmitoylphosphatidylglycerol, and dioleoylphosphatidylglycerol are used.

In preparing lipid-based vesicles containing an anionic compound, such variables as the efficiency of anionic compound encapsulation, lability of the anionic compound, homogeneity and size of the resulting population of vesicles, anionic compound-to-lipid ratio, permeability, instability of the preparation, and pharmaceutical acceptability of the formulation should be considered (see Szoka, et al., *Annual Reviews of Biophysics and Bioengineering*, 9:467, 1980; Deamer, et al., in *Liposomes*, Marcel Dekker, New York, 1983, 27; and Hope, et al., *Chem. Phys. Lipids*, 40:89, 1986, the contents of which are incorporated herein by reference).

Administration of the Pharmaceutically Acceptable Formulation

In one embodiment, the anionic compound is administered by introduction into the central nervous system of the subject, e.g., into the cerebrospinal fluid of the subject. In certain aspects of the invention, the anionic compound is introduced intrathecally, e.g., into a cerebral ventricle, the lumbar area, or the cisterna magna.

The pharmaceutically acceptable formulations can easily be suspended in aqueous vehicles and introduced through conventional hypodermic needles or using infusion pumps. Prior to introduction, the formulations can be sterilized with, preferably, gamma radiation or electron beam sterilization, described in US patent no. 436,742 the contents of which are incorporated herein by reference.

In another embodiment of the invention, the anionic compound formulation is administered into a subject intrathecally. As used herein, the term "intrathecal administration" is intended to include delivering an anionic compound formulation directly into the cerebrospinal fluid of a subject, by techniques including lateral

cerebroventricular injection through a burrhole or cisternal or lumbar puncture or the like (described in Lazorthes et al. Advances in Drug Delivery Systems and Applications in Neurosurgery, 143-192 and Omaya et al., Cancer Drug Delivery, 1: 169-179, the contents of which are incorporated herein by reference). The term "lumbar region" is intended to include the area between the third and fourth lumbar (lower back) vertebrae. The term "cisterna magna" is intended to include the area where the skull ends and the spinal cord begins at the back of the head. The term "cerebral ventricle" is intended to include the cavities in the brain that are continuous with the central canal of the spinal cord. Administration of an anionic compound to any of the above mentioned sites can be achieved by direct injection of the anionic compound formulation or by the use of infusion pumps. For injection, the anionic compound formulation of the invention can be formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the anionic compound formulation may be formulated in solid form and re-dissolved or suspended immediately prior to use. Lyophilized forms are also included. The injection can be, for example, in the form of a bolus injection or continuous infusion (e.g., using infusion pumps) of the anionic compound formulation.

Duration and Levels of Administration

In another embodiment of the method of the invention, the pharmaceutically acceptable formulation provides sustained delivery, e.g., "slow release" of the anionic compound to a subject for at least one, two, three, or four weeks after the pharmaceutically acceptable formulation is administered to the subject.

As used herein, the term "sustained delivery" is intended to include continual delivery of an anionic compound *in vivo* over a period of time following administration, preferably at least several days, a week or several weeks. Sustained delivery of the anionic compound can be demonstrated by, for example, the continued therapeutic effect of the anionic compound over time (*e.g.*, sustained delivery of the anionic compound can be demonstrated by continued inhibition of neuronal cell death over time). Alternatively, sustained delivery of the anionic compound may be demonstrated by detecting the presence of the anionic compound *in vivo* over time.

In one embodiment, the pharmaceutically acceptable formulation provides sustained delivery of the anionic compound to a subject for less than 30 days after the anionic compound is administered to the subject. For example, the pharmaceutically acceptable formulation, e.g., "slow release" formulation, can provide sustained delivery of the anionic compound to a subject for one, two, three or four weeks after the anionic

compound is administered to the subject. Alternatively, the pharmaceutically acceptable formulation may provide sustained delivery of the anionic compound to a subject for more than 30 days after the anionic compound is administered to the subject.

The pharmaceutical formulation, used in the method of the invention, contains a therapeutically effective amount of the anionic compound. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired result. A therapeutically effective amount of the anionic compound may vary according to factors such as the disease state, age, and weight of the subject, and the ability of the anionic compound (alone or in combination with one or more other agents) to elicit a desired response in the subject. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the anionic compound are outweighed by the therapeutically beneficial effects. A non-limiting range for a therapeutically effective concentration of an anionic compound is 100 mM to 1 mM. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the anionic compound and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed invention.

Preferred compounds of the invention include sulfates, sulfonates, phosphates, carboxylates, and compounds which include combinations of these functional groups. Particularly preferred compounds include substituted and unsubstituted lower alkyl sulfates and sulfonates (including without limitation, 1,4-butanediol disulfate, sodium 1,5-pentanedisulfonate, taurine (sodium 2-amino-ethanesulfonate), and homotaurine (3-aminopropanesulfonic acid). Other preferred compounds include 3-(cyclohexylamino)-1-propane sulfonate, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonate, 3-(N-morpholino)propanesulfonic acid, sodium tetrahydrothiophene-1,1-dioxide-3,4-disulfate trihydrate, sodium 4-hydroxybutane-1-sulfonate, sodium 1,3,5-pentanetriol trisulfate, 2-aminoethyl hydrogen sulfate, phosphonoformic acid, phosphonoacetic acid, or indigo carmine. A preferred compound is 3-aminopropanesulfonic acid, or a salt thereof (see Example, *infra*).

In another aspect, the invention provides a method for inhibiting an inflammatory process (e.g., an inflammatory process due to the presence of, or activation of macrophages by, an amyloidogenic protein or peptide). The method comprises administering to a subject in need thereof (e.g., a subject having amyloid deposition) an effect therapeutic amount of an anionic compound, such that the inflammatory process is

inhibited, e.g., by inhibition of macrophage activation by an amyloidogenic protein or peptide, such as Aβ. In a preferred embodiment, the subject is a subject suffering from Alzheimer's disease. In certain embodiments, the anionic compound is a compound represented by Formulas I or II. Preferred therapeutic compounds include sulfates, sulfonates, phosphates, carboxylates, and compounds which include combinations of these functional groups. Particularly preferred compounds include substituted and unsubstituted lower alkyl sulfates and sulfonates (including without limitation, 1,4-butanediol disulfate, sodium 1,5-pentanedisulfonate, taurine (sodium 2-amino-ethanesulfonate), and homotaurine (3-aminopropanesulfonic acid). Other preferred compounds include 3-(cyclohexylamino)-1-propane sulfonate, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonate, 3-(N-morpholino)propanesulfonic acid, sodium tetrahydrothiophene-1,1-dioxide-3,4-disulfate trihydrate, sodium 4-hydroxybutane-1-sulfonate, sodium 1,3,5-pentanetriol trisulfate, 2-aminoethyl hydrogen sulfate, phosphonoformic acid, phosphonoacetic acid, or indigo carmine. A preferred compound is 3-aminopropanesulfonic acid, or a salt thereof.

Example 1

Macrophages (Bone-marrow derived macrophages - RAW cells) were incubated in serum free medium with $A\beta_{1-40}$ fibrils ($A\beta_{1-40}$ is a polypeptide corresponding to residues 1-40 of the Aβ protein) (final concentration 2.5 μM) with or without the presence of lipopolysaccharide (LPS) (0.01 μg/ml) as a co-inducer of activation. The macrophages were incubated overnight. Supernatants were harvested and inflammatory cytokines TNFα, IL-6, as well as nitric oxide were measured. TNFα, IL-6 were measured by an ELISA, while NO was measured by Griess Reagent.

Negative controls consisted of cells incubated with LPS or Aβ alone. Positive control consisted of cells incubated with LPS and IFNγ at concentrations known to induce an optimal activation of these cells.

As shown in Figures 1 and 2, 3-aminopropanesulfonic acid (present in solution as the salt form) was found to block about 60% of the Aβ-induced TNFα production, while IL-6 production was not shown to be affected. NO production was also shown to be inhibited by 3-aminopropanesulfonic acid, while this compound did not appear to have any significant effect on the TNFα and NO produced by RAW cells in presence of LPS and IFNγ.

The contents of all references, issued patents, and published patent applications cited throughout this application, including the background, are hereby incorporated by reference.

Example 2

The following example demonstrates the ability of compounds of the invention to inhibit Aβ-induced microglia activation.

Human microglia THP-1 cells were primed with LPS (lipopolysaccharide) (0.25 μg/ml) and then incubated with a 5 uM preparation of fibrillary Aβ peptide. Activation was determined by measuring the amount of IL-1β released in the cell culture supernatant. The ability of a compound to block/inhibit the activation process was determined by comparing the amount of cytokine (here, IL-1β) present in the supernatant when cells were incubated with a compound to that obtained in the supernatant of control cells (incubated with Aβ).

When cells were treated with a sulfonated compound, here, 3-aminopropanesulfonic acid, a significant decrease (shown in FIG. 3) in the amount of IL-1β was seen at concentration of 10^{-7}M to 10^{-3}M, indicating inhibition of microglia.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.

What is claimed is:

1. A method for inhibiting macrophage activation by an amyloidogenic protein or peptide, comprising:

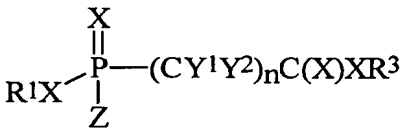
contacting a macrophage in the presence of an amyloidogenic protein or peptide with an anionic compound, such that macrophage activation is inhibited.

2. The method of claim 1 wherein said anionic compound has the structure

$$Q—[—Y^-X^+]_n$$

wherein Y^- is an anionic group at physiological pH; Q is a carrier molecule; X^+ is a cationic group; and n is an integer selected such that the biodistribution of the compound for an intended target site is not prevented while maintaining activity of said compound.

3. The method of claim 1 wherein said anionic compound has the structure:



wherein Z is XR^2 or R^4, R^1 and R^2 are each independently hydrogen, a substituted or unsubstituted aliphatic group, an aryl group, a heterocyclic group, or a salt-forming cation; R^3 is hydrogen, lower alkyl, aryl, or a salt-forming cation; R^4 is hydrogen, lower alkyl, aryl or amino; X is, independently for each occurrence, O or S; Y^1 and Y^2 are each independently hydrogen, halogen, alkyl, amino, hydroxy, alkoxy, or aryloxy; and n is an integer from 0 to 12.

4. The method of claim 1 wherein said anionic compound is selected from the group consisting of 1,4-butanediol disulfate, sodium 1;5-pentanedisulfonate, taurine (sodium 2-amino-ethanesulfonate), homotaurine (3-aminopropanesulfonic acid). 3-(cyclohexylamino)-1-propane sulfonate, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonate, 3-(N-morpholino)propanesulfonic acid, sodium tetrahydrothiophene-1,1-dioxide-3,4-disulfate trihydrate, sodium 4-hydroxybutane-1-sulfonate, sodium 1,3,5-pentanetriol trisulfate, 2-aminoethyl hydrogen sulfate, phosphonoformic acid, phosphonoacetic acid, indigo carmine, pharmaceutically acceptable salts thereof.

5. The method of claim 1, wherein the anionic compound is 3-aminopropanesulfonic acid, or a pharmaceutically acceptable salt thereof.

6. A method for inhibiting an inflammatory process, comprising:

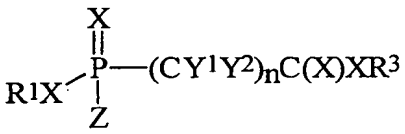
administering to a subject in need thereof an effect therapeutic amount of an anionic compound, such that the inflammatory process is inhibited.

7. The method of claim 6 wherein said anionic compound has the structure

$$Q—[—Y^-X^+]_n$$

wherein Y^- is an anionic group at physiological pH; Q is a carrier molecule; X^+ is a cationic group; and n is an integer selected such that the biodistribution of the compound for an intended target site is not prevented while maintaining activity of said compound.

8. The method of claim 6 wherein said anionic compound has the structure:



wherein Z is XR^2 or R^4, R^1 and R^2 are each independently hydrogen, a substituted or unsubstituted aliphatic group, an aryl group, a heterocyclic group, or a salt-forming cation; R^3 is hydrogen, lower alkyl, aryl, or a salt-forming cation; R^4 is hydrogen, lower alkyl, aryl or amino; X is, independently for each occurrence, O or S; Y^1 and Y^2 are each independently hydrogen, halogen, alkyl, amino, hydroxy, alkoxy, or aryloxy; and n is an integer from 0 to 12.

9. The method of claim 6 wherein said anionic compound is selected from the group consisting of 1,4-butanediol disulfate, sodium 1,5-pentanedisulfonate, taurine (sodium 2-amino-ethanesulfonate), homotaurine (3-aminopropanesulfonic acid). 3-(cyclohexylamino)-1-propane sulfonate, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonate, 3-(N-morpholino)propanesulfonic acid, sodium tetrahydrothiophene-1,1-dioxide-3,4-disulfate trihydrate, sodium 4-hydroxybutane-1-sulfonate, sodium 1,3,5-pentanetriol trisulfate, 2-aminoethyl hydrogen sulfate, phosphonoformic acid, phosphonoacetic acid, indigo carmine, pharmaceutically acceptable salts thereof.

10. The method of claim 9, wherein the anionic compound is 3-aminopropanesulfonic acid, or a salt thereof.

11. The method of claim 9, wherein the subject is a subject suffering from Alzheimer's disease.

12. The method of claim 11, wherein the inflammatory process is due to activation of macrophages by an amyloidogenic protein or peptide, or a fragment thereof.

13. The method of claim 12, wherein the amyloidogenic protein or peptide is Aβ.

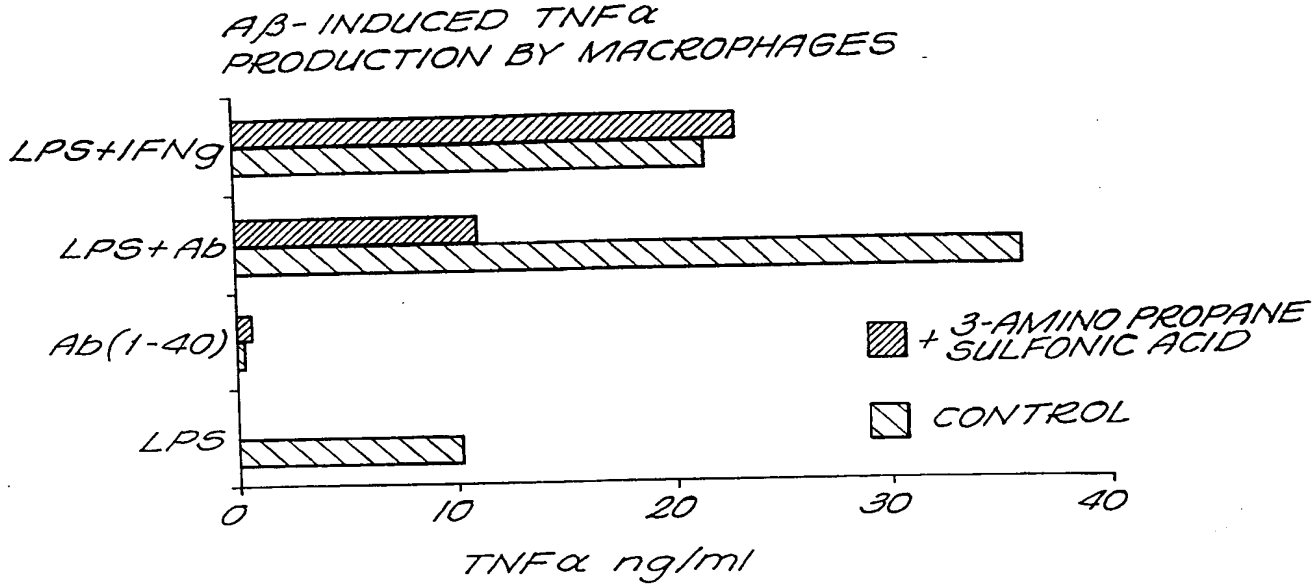


FIG. 1

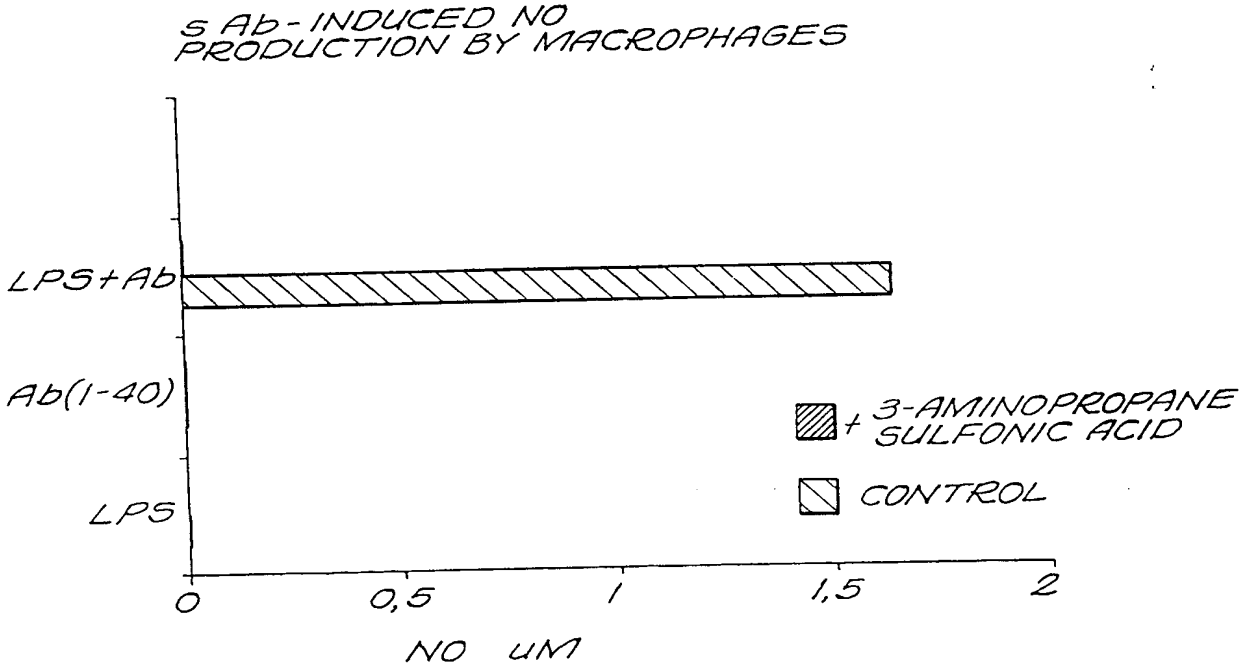


FIG. 2

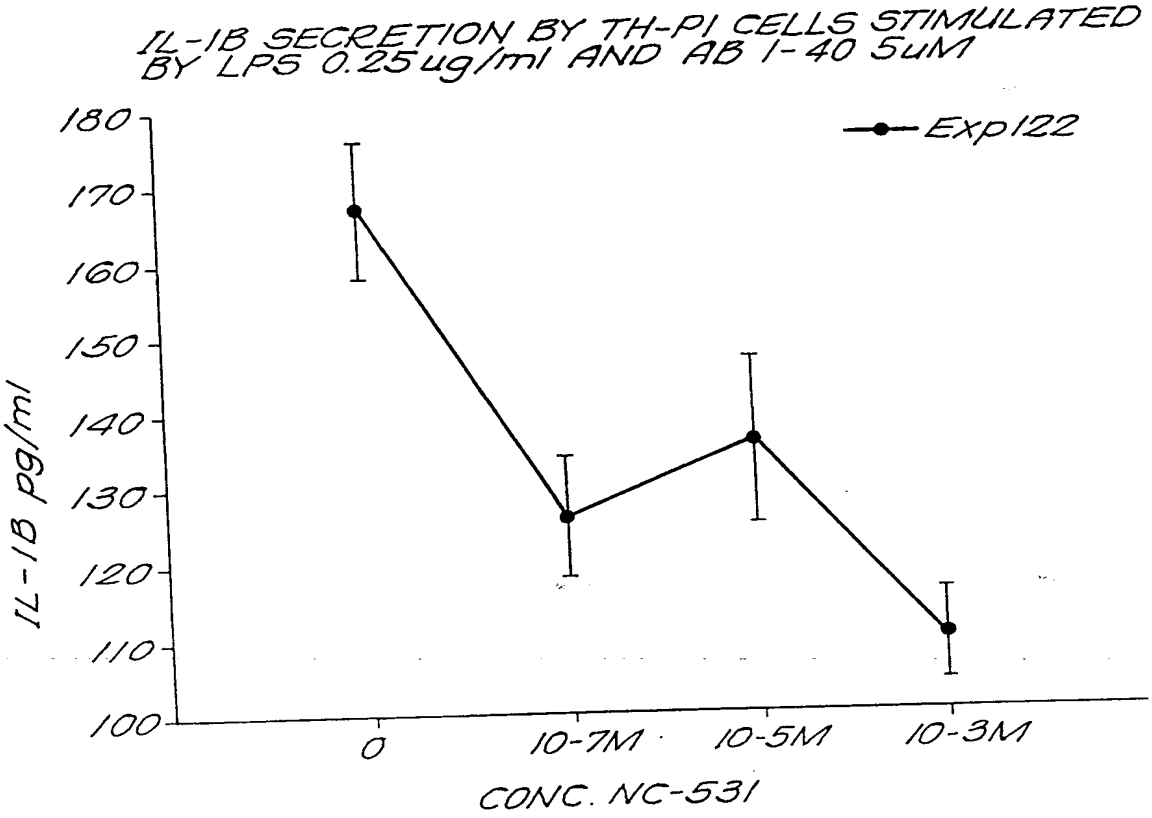


FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 99/00354

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/185 A61K31/255 A61K31/66 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 99 08685 A (QUEEN'S UNIVERSITY AT KINGSTON) 25 February 1999 see claims 1-33 ---	1-4,6-9, 11-13
X,P	US 5 869 469 A (W. A. SCZAREK ET AL) 9 February 1999 see the whole document ---	1-4,6-9, 11-13
X	WO 96 28187 A (QUEEN'S UNIVERSITY AT KINGSTON) 19 September 1996 see claims 1-19 see page 5, line 1 - page 17, line 11 & US 5 643 562 A cited in the application --- -/--	1,2,4-7, 9-13

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 June 1999	11/06/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Siatou, E

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	KISILEVSKY R ET AL: "ARRESTING AMYLOIDOSIS IN VIVO USING SMALL-MOLECULE ANIONIC SULPHONATES OR SULPHATES: IMPLICATIONS FOR ALZHEIMER'S DISEASE" NATURE MEDICINE, vol. 1, no. 2, 1 February 1995, pages 143-148, XP000611547 see the whole document	1,2,4,6, 7,9, 11-13
X	WO 94 22437 A (QUEEN'S UNIVERSITY AT KINGSTON) 13 October 1994 see claims 1-47	1,2,4,6, 7,9, 11-13
X	US 5 276 059 A (B. CAUGHEY ET AL) 4 January 1994 see claims 9-34 see column 2, line 14 - line 24 see table 1	1,2,6,7
A	US 4 386 081 A (A. J. E. HELGSTRAND ET AL) 31 May 1983 see the whole document	1-13
A	US 5 463 092 A (K. Y. HOSTETLER ET AL) 31 October 1995 see the whole document	1-13
A	US 5 389 623 A (N. S. BODOR) 14 February 1995 cited in the application see claims 1-28	1-13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 99/00354

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-13
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-13 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

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